

FURTHER REMARKS UPON THE MECHANISM OF AGGLUTINATION.

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Two years ago, at the April meeting of this Society, I read a paper* upon the mechanism of agglutination, in which I reviewed the theories regarding the phenomenon which had been published up to that time. I also gave, as the result of my work and experience, the hypothesis that the agglutination of bacteria is due to the formation of a chemical precipitate upon the bacteria through the interaction of the agglutinating substance of the active serum with the agglutinable substance secreted by the bacteria. The chemical precipitate upon the bacteria is flocculated by the salts of the serum or of the bouillon, and the bacteria are carried with the precipitate mechanically. My hypothesis differed from that of Nicolle and of Paltauf by taking into account the function of the salts and the mechanical part played by the organisms.

Since the publication of my paper two authors have published papers detailing experiments which show the function of salts in promoting agglutination, but they miss the point that it is *the precipitate on the bacteria that is agglutinated*, clumped, or flocculated by the salts.

I was the first to emphasise the necessary rôle of the salts in explaining the mechanism of the process; and although Bordet

* Proc. Linn. Soc. N.S.W. xxv., 75. Referat in Cent. f. Bakt. 1 Abt. xxx., 209.

had published an experiment showing how common salt was necessary in causing an agglutination, he failed to accentuate the part played by salts. Although most writers upon the subject agree in considering agglutination as a chemical phenomenon, they do not understand, or at least fail to indicate, the steps in the process. Were it otherwise, the recently published papers upon the rôle of salts in agglutination would probably not have been written.

The first of these papers is by Joos,* who, after enumerating his experiments, concluded that—

1. No agglutination takes place when the agglutinating substance acts upon the agglutinable substance in the absence of common salt.
2. Agglutination always occurs when the three substances are together, viz., the agglutinating substance, the agglutinable substance and salt.
3. In the absence of salt the agglutinating substance is quickly combined with the agglutinable substance of the bacteria. This combination alters their vitality in no way.
4. There is a close relation between the relative amounts of substances which together bring about the phenomenon of agglutination and of the obtained quantity of agglutinated substance.
5. Salt plays an active rôle in the phenomenon.
6. Salt enters into the combination of the agglutinating and agglutinable substance.
7. Agglutination can occur in a salt-free medium if the bacteria contain salt.
8. The "physical theory" of Bordet is untenable.

These conclusions of Joos simply bear out the contention that agglutination depends upon the flocculating action of a particular salt, viz., common salt, for the reason that this was the only salt with which he experimented.

* Joos, *Zeit. f. Hygiene*, xxxvi., 422.

A year afterwards Friedberger* published a paper in which he showed that other salts were as useful as common salt in causing agglutination. He concluded that :—

1. Agglutination does not occur in the complete absence of crystalline substances.
2. Of these substances the inorganic salts are the most active and they differ among themselves in their activity.
3. The rapidity of the onset of the agglutination of dialysed cultures depends upon the salt content of the bacterial suspension.
4. The rapidity of the onset of the agglutination of a bacterial emulsion depends upon its salt content.
5. The action of salts in agglutination is not chemical.

Thus the function of salts in general in causing agglutination is emphasised. Exception must, however, be taken to the 5th item, which is rather startling. Friedberger does not attempt to explain the mechanism of the process, and that he cannot understand the part played by the salts is made evident by his sweeping assertion. By chemical, he means that the salts do not chemically combine with the albumen of the cell, an idea which has never been advanced with any positiveness. Bordet, perhaps by considering that Kraus' precipitate did not play a part in agglutination, suggested the idea of an enzyme altering an albuminoid constituent of the microbic cell; but beyond this inference there is nothing to justify the assumption that coagulation of the cell albuminoids may occur. That the bacteria simply become immobilised is well known. Were a portion of their albuminoid matter coagulated, probably something more than immobilisation would ensue. Joos confirmed the fact that agglutination does not alter the vitality of the bacteria.

In a later paper Joos,† after pointing out that Friedberger's researches strengthened his own former conclusions, finally pro-

* Friedberger, *Cent. f. Bakt.* xxx., 336.

† Joos, *Cent. f. Bakt.* 1 Abt. xxx., 853.

pounded the following as an explanation of the mechanism:—

The phenomenon of agglutination is made up of two quite distinct phases. In the first the specific substances combine with the salts. The second is that in which the microbes modified by this combination unite into floccules.

The first phase depends upon a chemical combination analogous to the formation of double salts, or of certain additive compounds. It does not depend upon a molecular attraction [Juxtaposition] as in dyeing or porosity phenomena.

The second phase is entirely similar to a chemical precipitate, and does not depend exclusively upon a disturbance of the molecular equilibrium of the mixture, which is produced by the solution of the salt.

These statements are vague, and we do not gather from them whether the individual specific substances (agglutinating and agglutinable) unite with the salts and then combine, or combine and then unite—an important point. Nor do we see how the microbes are modified by this combination of salt and specific substance. Nor is the relation between the microbes and the combined specific substance defined. Although this is an attempt to explain the mechanism by bringing the phenomenon into line with the formation of chemical precipitates, the mechanism itself is still unexplained. That the phenomenon is different from that which occurs in staining fibres, etc., may be open to doubt, for Knecht (*Ber.* 1902, 35, 1022) has shown that dyeing depends upon the formation of lakes between the colour and certain constituents of wool and silk.

On the whole there is a tendency in these papers to consider the phenomenon in a manner that is too narrow and too indefinite to be scientific. That agglutination is similar to coagulation and precipitation appears to be admitted by most writers, *e.g.*, Bordet, Duclaux, Nicolle, Paltauf, Köhler and Joos; but the mechanism of coagulation does not appear to be understood, or Joos would probably not have emphasised the rôle of common salt in agglutination, and Bordet would have mentioned the function of calcium

salts in the coagulation of casein, to which he likened agglutination.

I have already* explained the mechanism of flocculation, coagulation or precipitation by the hydrate theory of solution, and although I understand that it has also been explained by the ionisation theory, yet I was, and still am, not satisfied with the latter theory as being a good one for the purpose.

The mechanism of agglutination is explained in the following manner by the hydrate theory of solution. The unknown agglutinating substance of the serum and the obscure agglutinable product of microbic metabolism are each dissolved in their respective fluids by reason of the "residual affinities" which they possess for loose water molecules. (In explanation of these terms it may be said that residual affinity is a chemical affinity of a weak kind, and that a water solution consists of molecular aggregates and loose water molecules.) Each molecule of the active substances is the centre of a system, and is surrounded by a zone of water molecules. The solutions are in equilibrium until they are mixed, when the active substances combine, the result of chemical affinity. Whether or not a decomposition also takes place we are unable to say, and at present this question may be ignored. The combined or double molecule now becomes the centre of a new water system, but its residual affinity for the loose water molecules is weaker than the residual affinity of the saline matter in the fluid. The origin of the saline matter is immaterial. The salts attract the water molecules, and withdraw them from the influence of the double molecule. The residual affinities of the double molecules are thus free, and a kind of molecular tension is produced. The double molecules are then attracted to one another and become molecular aggregates, which gradually increase in complexity, while the equilibriums between aggregate and solution are constantly altering. Eventually the aggregates grow so massive that they become visible as a precipitate. This is Kraus' precipitate. When substances separate out from solution they

* Jour. Soc. Chem. Ind. xvii., 117.

tend to form upon small solid particles just as rain-drops gather around dust particles, crystals form around string, and even precipitates adhere to glass. Thus Kraus' precipitate forms upon the flagella and upon the bacterial cell. It forms upon the bacteria the more readily since the capsule is saturated with the agglutinable substance which slowly oozes through the membrane. But the building up of the molecular aggregates still proceeds, and the precipitated films surrounding the bacteria are attracted to one another, so that small clumps are formed and agglutination is made manifest, because we can see the comparatively large bacteria. The thin precipitated films elude our observation.

Although the residual affinity alone has been used to explain the phenomenon, yet the base of the flocculating salt over and above its attraction for the loose water molecules plays a part in combining with the molecular aggregates. The base partly attracts the water molecules, and partly replaces them. Even some of the acid radicles may in some instances be carried down (attracted) with the precipitate as in the coagulation of slimes and mucin bodies by salts in alcoholic solution.

Agglutination is a chemical phenomenon, and the natural laws which govern it are those which obtain in the precipitation of chemical compounds, the coagulation of precipitates, the flocculation of clay particles, and even the solution and diffusion of substances in water. The bodies of the bacteria have tended to obscure the mechanism of the process, a thing which should not have been done in view of what Nicolle did when he agglutinated particles of talc suspended in filtered typhoid cultures.

A paper has recently been written by Harrison* upon the agglutinating substance of typhoid bacteria. He took typhoid bacteria which agglutinated readily with active sera, and treated them with with pyocyanase, an enzyme which has the power of dissolving the outer membranes of bacteria. After washing the membraneless bacteria with water, he found that they were not now capable of being agglutinated. From this he concluded

* Harrison, *Cent. f. Bakt.* 1 Abt. xxx., 115.

that the agglutinable substance existed "entirely in the outer layers of the bacilli."

Jochmann,* in abstracting a paper by Köhler, remarked that Harrison had in a very clear manner proved Nicolle's assumption that the agglutinable substance is found in the outer membranes of the bacteria. With this I do not agree. The function of a cell membrane is to retain the cell contents, and the membrane of the typhoid bacterium retains the agglutinable substance to a considerable extent. That it slowly diffuses through is admitted from the scanty formation of Kraus' precipitate in younger cultures, and the more copious precipitation in older cultures. But if we remove the capsule or membrane there is no restraint put upon the diffusion of the agglutinable substance from *within* the cell. Harrison's experiments simply show that by removing the membrane or capsule one of the slowly diffusible constituents of the cell can be quickly removed, a circumstance which no one would have doubted. They do *not* prove that the agglutinable substance is in the surface layers of the cell. One might remove the covering from a bee-hive, and with the same amount of reason say that the bees which swarm out were in the covering, or when an egg is broken that all the fluid contents were originally in the substance of the shell. No! The agglutinable substance saturates the bacterium as a whole; there is no evidence to show that it is otherwise.

* Jochmann, Cent. f. Bakt. 1 Abt. xxx. 585.